



Health Alert Network

Tri-County Health Department

Serving Adams, Arapahoe and Douglas Counties

Phone 303/220-9200 • Fax 303/741-4173 • www.tchd.org

Follow us on Twitter @TCHDHealth and @TCHDEmergency

John M. Douglas, Jr., M.D. Executive Director

The pages that follow contain information critical to protecting the health of your patients and the citizens of Colorado.

HAN UPDATE

Number of pages including cover: 11

Subject: **Update - Recommendations for Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection**

Message ID: 6/3/2016 4:15:00 PM

Recipients: HAN Community Members.

From: TRI-COUNTY HEALTH DEPARTMENT

Adams, Arapahoe and Douglas County, Colorado

Recipient Instructions: **Tri-County Health Department is sending you the attached HAN. You may have received a similar broadcast from CDPHE, however, we have included additional updates/clarification. Thank you. No response is required.**

=====

You have received this message based upon the information contained within our Health Alert Network Notification System. If you have a different or additional e-mail or fax address that you would like us to use, or if you have additional questions, call 720-200-1477.

Categories of Health Alert Network Messages:

Health Alert: Conveys the highest level of importance; warrants immediate action or attention.

Health Advisory: Provides important information for a specific incident or situation; may not require immediate action.

Health Update: Provides updated information regarding an incident or situation; unlikely to require immediate action.

Info Service/Public Health Brief: Provides general information that is not necessarily considered to be of an emergent nature.

You may download a copy of this HAN from the TCHD website at
<http://www.tchd.org/259/Health-Alert-Network>



HEALTH UPDATE

Recommendations for Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection
June 2, 2016

****Health care providers: Please distribute widely in your office****

KEY POINTS:

This is an evolving situation.

Recommendations will be updated as more information becomes available.

- The CDC Trioplex rRT-PCR assay is authorized by FDA for Zika virus testing of urine and serum. This PCR assay detects virus in clinical samples, and is *only useful for symptomatic patients*.
- **New Recommendation:** Collect and submit urine samples within 14 days of symptom onset *along with* patient-matched serum samples for those who meet CDC Zika virus clinical and/or epidemiological testing criteria for Zika virus infection.

Based on when a patient presents:

- Submit urine and serum for RT-PCR testing from first day of symptoms through day 7 following symptom onset
- Submit urine for RT-PCR and serum for IgM testing from day 7 through day 14 following symptom onset
- Submit serum only for IgM testing after day 14 following symptom onset
- In Colorado, the State Laboratory can perform Zika virus RT-PCR testing on serum and urine.
- Please consult CDPHE for testing assistance: 303-692-2700 (business hours) or 303-370-9395 (after hours, weekends and holidays). The CDPHE Laboratory can be reached at 303-692-3485 (Serology) or 303-692-3286 (Molecular Sciences).
- Patient specimens should be collected using typical healthcare resources. CDPHE does not provide patient specimen collection services.
- REFER TO HAN ATTACHMENTS for details about ordering Zika testing and interpreting Zika RT-PCR and IgM results

Attachments:

- 1) CDPHE: Q & A describes how to order Zika Specimen Testing
- 2) CDC HAN Health Update: Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection. May 25, 2016
- 3) CDC Interim Guidance for Interpretation of Zika Virus Antibody Test Results, MMWR Early Release May 31, 2016

FOR MORE INFORMATION:

Zika virus information for clinicians: <http://www.cdc.gov/zika/hc-providers/index.html>



Q & A's: Zika Virus Diagnostic Testing

June 2, 2016

This is an evolving situation.

Recommendations will be updated as more information becomes available.

Who should be tested for Zika virus?

Pregnant women who traveled to a Zika-affected area:

- Who had one or more of the following symptoms during or within two weeks of travel: acute onset of fever, rash, arthralgia or conjunctivitis.
- Who traveled within the past two to 12 weeks and had no symptoms of Zika.
- During the eight weeks before conception can be tested within two to 12 weeks of that exposure.

Pregnant women who did *not* travel to a Zika-affected area:

- Who had unprotected sexual contact with a male partner who traveled to a Zika-affected area, where the male partner was diagnosed with, or had symptoms of Zika infection.
- Who became symptomatic after having unprotected sexual contact with a male partner who traveled to a Zika-affected area.

All others:

- Who traveled to a Zika-affected area and had one or more symptoms of Zika virus infection during or within two weeks of travel.
- Who became symptomatic after unprotected sexual contact with a male partner who traveled to a Zika-affected area, where the male partner was diagnosed with, or had symptoms of Zika infection.

*Men and non-pregnant women who are asymptomatic are not being tested at this time.

What testing is available and where?

PCR:

- Triplex RT-PCR Assay to detect dengue, chikungunya and Zika virus RNA [available at the state health lab].
- RealStar RT-PCR to detect Zika virus RNA [available through commercial labs].

Serology:

- IgM capture (MAC) ELISA to detect Zika virus IgM [available at CDC].
- Plaque Reduction Neutralization Test (PRNT) to detect total antibody (dengue and Zika virus) [available at CDC; performed on IgM positive or equivocal samples].
- Dengue MAC-ELISA and chikungunya MAC-ELISA [available through commercial labs].

How can patients access Zika virus testing?

Zika virus testing is only available when requested by a health care provider. The state health lab cannot collect samples directly from patients.

What samples can be tested for Zika virus?

For symptomatic patients:

- Collect urine and serum for RT-PCR testing from the first day of symptoms through day 7 following symptom onset.

- Collect urine for RT-PCR testing and serum for serology testing from day 7 through day 14 following symptom onset.
- Collect CSF or amniotic fluid for RT-PCR testing from the first day of symptoms through day 7 following symptom onset.
- IgM testing can also be requested on serum, CSF and amniotic fluid samples ≥ 4 days following symptom onset.

For asymptomatic pregnant women:

All testing is serological and currently performed at CDC. MAC-ELISA is available, and serum and amniotic fluid can be tested. Collect samples two to 12 weeks following travel to a Zika-affected area.

What samples should be collected and how much?

Serum:

- Use a red top, tiger top or serum separator tube.
- The state health lab requires 0.25 mL for RT-PCR testing.
- CDC requires ≥ 1.0 mL of serum for IgM testing.
- If requesting both RT-PCR and IgM testing, send ≥ 1.25 mL.

Urine:

- Send the sample in a sterile, screw-capped vial secured with thermoplastic, self-sealing lab film.
- The state health lab requires 0.5 to 1.0 mL for RT-PCR testing.

Amniotic fluid:

- Send sample in a sterile, screw-capped vial secured with thermoplastic, self-sealing lab film.
- CDC requires 0.5 to 1.0 mL for RT-PCR and/or IgM testing.

Cerebrospinal fluid (CSF):

- Send sample in a sterile, screw-capped vial secured with thermoplastic, self-sealing lab film.
- CDC requires at least 1.0 ml for RT-PCR or IgM testing.

What is the turnaround time for Zika testing?

- RT-PCR testing at the state health lab will take approximately 72 hours following sample receipt.
- Serology testing at CDC will take approximately 4 - 6 weeks.
- All results will be sent securely to the submitter at the fax number provided.

How do I request testing from the lab?

All samples need to be sent directly to the state health lab. The lab will not determine which test to perform, nor will it automatically send samples for CDC testing unless indicated.

All submitters **MUST** submit the Request for Analytical Services form #270/271. A separate form must be filled out for EACH sample.

- To request the form, call the state health lab (303-692-3485) or state health department (303-692-2700).
- The lab will use the form to set up an account for you if you do not already have one.

Submitters requesting IgM testing must also complete the CDC 50-34 form.

- The form is available at www.cdc.gov/laboratory/specimen-submission/pdf/form-50-34.pdf, or

- Call 303-692-2700 and we can fax or email the form to you.

The CDC 50-34 form *must be filled out completely* or testing will be delayed.

- In the ‘Test order name’ field on page 1 of the form, put *ZIKA*.
- On page 2 under ‘Relevant immunization history’ indicate if patient has a history of yellow fever or Japanese encephalitis virus vaccination.

What is the cost for testing, and is there a CPT code?

- The RT-PCR test at the state health lab costs \$260 (per sample), billed to the submitter [CPT code 87798].
- There is a \$45 handling and processing fee for IgM testing at CDC [CPT code 99001].

How do I get the sample to the state health lab?

- Specimens can be sent to the state health lab by FedEx or by the courier of your choice. The address is CDPHE Laboratory Services Division, 8100 Lowry Blvd, Denver, CO 80230.
 - The state health lab can only accept samples Monday - Friday, 8 a.m. to 5 p.m.
 - FedEx and other couriers should be directed to avoid weekend deliveries.
- Alternately, you may check our current list of pickup times and locations for the state health lab courier,
www.colorado.gov/pacific/sites/default/files/Kangaroo%20Update.7.29.14-SK.pdf
 - If your hospital lab or local health department is on the courier route, you may arrange to have the sample(s) picked up there. Call 303-692-3086 to make arrangements.

Are there special handling and packaging instructions?

Samples should be kept at 4°C and transported on cold packs. The CDPHE courier will transport at 4°C; other overnight shipments should include a cold pack. If the sample is frozen, it should be kept frozen and be shipped on dry ice.

Can samples be submitted to commercial labs for testing?

Under the FDA’s Emergency Use Authorization, some commercial labs are performing Zika virus RT-PCR testing. For more information on submitting samples to commercial labs, contact your regular commercial laboratory.

Who can I call for other questions about Zika virus?

Call the state health lab at 303-692-3485 or the state health department at 303-692-2700.

Where can I go for more information on Zika virus?

A list of countries with local transmission of Zika virus is available at www.cdc.gov/zika/geo/active-countries.html

The most up to date testing guidance is available at www.colorado.gov/pacific/cdphe/zika

This is an official

HAN HEALTH UPDATE

Distributed via the CDC Health Alert Network
May 25, 2016, 14:15 EDT (2:15 PM EDT)
CDCHAN-00389

Diagnostic Testing of Urine Specimens for Suspected Zika Virus Infection

Summary

On May 13, 2016, the Centers for Disease Control and Prevention (CDC) issued [interim guidance](http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e1.htm) (<http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e1.htm>) that recommends Zika virus rRT-PCR testing of urine collected less than 14 days after symptom onset, along with testing of patient-matched serum samples, for the diagnosis of suspected Zika virus infection (1). The purpose of this Health Alert Network (HAN) health update is to further disseminate information about the interim guidance to clinical and public health professionals.

Background

Zika virus is a mosquito-borne flavivirus. Zika virus infection during pregnancy can cause microcephaly and other severe fetal brain defects. Zika virus infection is also associated with Guillain-Barré syndrome. Transmission of Zika can occur through mosquito bite, from a pregnant woman to her fetus, through sexual contact with an infected male, and possibly through blood transfusion. The most common symptoms of Zika virus disease are fever, rash, joint pain, or conjunctivitis. Other common symptoms include muscle pain and headache. Evidence from case reports and experience from related flavivirus infections indicate that the incubation period for Zika is likely a few to 14 days.

Diagnostic testing for Zika virus infection can be accomplished using molecular and serologic methods. The U.S. Food and Drug Administration (FDA) has issued [Emergency Use Authorizations](http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm) (EUA) (<http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>) for several diagnostic assays to detect Zika virus infection (2). The EUAs authorize real-time reverse transcription-polymerase chain reaction (rRT-PCR) assays to detect Zika virus RNA in specified clinical sample types, and an immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assay (ELISA) to detect anti-Zika virus IgM antibodies in serum and cerebrospinal fluid. The CDC Trioplex rRT-PCR assay is authorized by FDA for Zika virus testing of urine and serum. Anti-Zika IgM antibodies develop during the first week of illness and persist for approximately 12 weeks following infection. However, extensive cross-reactivity can occur in flavivirus serological assays, and therefore additional tests, such as the plaque reduction neutralization test (PRNT), are necessary to distinguish Zika virus infection from other flavivirus infections.

Although Zika virus RNA is unlikely to be detected in serum after the first week of illness, recent data suggest that Zika virus RNA can persist in urine for at least two weeks post symptom onset (3). Given this information, on May 13, 2016, CDC issued interim guidance on rRT-PCR testing for Zika virus RNA in urine (1). CDC now recommends that, for persons with suspected Zika virus disease, Zika virus rRT-PCR should be performed on both urine and serum specimens collected within 7 days after onset of symptoms. Zika virus rRT-PCR also should be performed on urine specimens collected within 14 days after onset of symptoms. A positive rRT-PCR result in either specimen confirms Zika virus infection. However, a negative rRT-PCR in a serum or urine sample collected at any time point after illness onset does not exclude Zika virus infection, and in these cases IgM antibody testing should be performed on serum.

CDC recommendations for Zika virus testing of serum and other clinical specimens remain unchanged at this time. Please contact your state or local health department to facilitate testing.

Recommendations for Health Care Providers and Public Health Practitioners

- Collect urine samples within 14 days post symptom onset along with patient-matched serum samples for those who match CDC Zika virus clinical and/or epidemiological testing criteria for Zika virus infection.
- Perform Zika virus rRT-PCR testing on urine, in conjunction with testing of serum using the appropriate molecular or serologic assay, based on days post symptom onset.

Additional Considerations

- Further investigation is needed to determine the sensitivity and utility of Zika virus rRT-PCR on urine specimens collected ≥ 14 days after onset of symptoms: limited data in pregnant women suggest that viremia in serum might be prolonged in pregnancy (4, 5).

References

1. CDC. Interim guidance for Zika virus testing of Urine – United States, 2016. MMWR Morb Mortal Wkly Rep 2016; 65. DOI: <http://dx.doi.org/10.15585/mmwr.mm6518e1>.
2. Food and Drug Administration. Emergency Use Authorizations. <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>
3. Comparison of Test Results for Zika Virus RNA in Urine, Serum, and Saliva Specimens from Persons with Travel-Associated Zika Virus Disease — Florida, 2016 <http://www.cdc.gov/mmwr/volumes/65/wr/mm6518e2.htm>
4. Driggers RW, Ho CY, Korhonen EM, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. N Engl J Med. March 30, 2016. DOI: 10.1056/NEJMoa1601824
5. Bocanegra C. Zika virus infection in pregnant women in Barcelona, Spain. Clin Microbiol Infect. April 3, 2016. DOI: 10.1016/j.cmi.2016.03.025.

For More Information

- General information about Zika virus and disease: <http://www.cdc.gov/zika/>
- Zika virus information for clinicians: <http://www.cdc.gov/zika/hc-providers/index.html>
- Memorandum – Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories (not updated with urine guidance): <http://www.cdc.gov/zika/state-labs/index.html>
- Diagnostic testing: <http://www.cdc.gov/zika/hc-providers/diagnostic.html>
- Collection and submission of body fluids for Zika virus testing: <http://www.cdc.gov/zika/hc-providers/body-fluids-collection-submission.html>

The Centers for Disease Control and Prevention (CDC) protects people's health and safety by preventing and controlling diseases and injuries; enhances health decisions by providing credible information on critical health issues; and promotes healthy living through strong partnerships with local, national, and international organizations.

Categories of Health Alert Network messages:

Health Alert Requires immediate action or attention; highest level of importance

Health Advisory May not require immediate action; provides important information for a specific incident or situation

Health Update Unlikely to require immediate action; provides updated information regarding an incident or situation

HAN Info Service Does not require immediate action; provides general public health information

This message was distributed to state and local health officers, state and local epidemiologists, state and local laboratory directors, public information officers, HAN coordinators, and clinician organizations

Interim Guidance for Interpretation of Zika Virus Antibody Test Results

Ingrid B. Rabe, MBChB¹; J. Erin Staples, MD, PhD¹; Julie Villanueva, PhD¹; Kimberly B. Hummel, PhD¹; Jeffrey A. Johnson, PhD¹; Laura Rose, MTS¹; Susan Hills, MBBS¹; Annemarie Wasley, ScD¹; Marc Fischer, MD¹; Ann M. Powers, PhD¹

Zika virus is a single-stranded RNA virus in the genus *Flavivirus* and is closely related to dengue, West Nile, Japanese encephalitis, and yellow fever viruses (1,2). Among flaviviruses, Zika and dengue virus share similar symptoms of infection, transmission cycles, and geographic distribution. Diagnostic testing for Zika virus infection can be accomplished using both molecular and serologic methods. For persons with suspected Zika virus disease, a positive real-time reverse transcription–polymerase chain reaction (rRT-PCR) result confirms Zika virus infection, but a negative rRT-PCR result does not exclude infection (3–7). In these cases, immunoglobulin (Ig) M and neutralizing antibody testing can identify additional recent Zika virus infections (6,7). However, Zika virus antibody test results can be difficult to interpret because of cross-reactivity with other flaviviruses, which can preclude identification of the specific infecting virus, especially when the person previously was infected with or vaccinated against a related flavivirus (8). This is important because the results of Zika and dengue virus testing will guide clinical management. Pregnant women with laboratory evidence of Zika virus infection should be evaluated and managed for possible adverse pregnancy outcomes and be reported to the U.S. Zika Pregnancy Registry or the Puerto Rico Zika Active Pregnancy Surveillance System for clinical follow-up (9,10). All patients with clinically suspected dengue should have proper management to reduce the risk for hemorrhage and shock (11). If serologic testing indicates recent flavivirus infection that could be caused by either Zika or dengue virus, patients should be clinically managed for both infections because they might have been infected with either virus.

Zika Virus Infection and Immune Response

Most Zika virus infections are asymptomatic (12). Viremia is expected to occur from several days before illness onset until a week after illness onset (6,13,14). Zika virus–specific

IgM antibodies develop during the first week of illness (5,6). Data on duration of IgM antibody persistence following Zika virus infection are limited. However, IgM antibodies against West Nile virus, a closely related flavivirus, have been detected in asymptomatic, infected blood donors for at least 3 months after their viremic donation, and almost half of tested patients with West Nile virus neuroinvasive disease had detectable serum IgM antibodies >1 year after illness onset (15,16). Neutralizing antibodies to Zika virus develop shortly after IgM antibodies and consist primarily of IgG antibodies. Neutralizing antibodies are expected to persist for many years after flavivirus infections and are believed to confer prolonged, possibly lifelong, immunity (17–19). In persons previously infected with a flavivirus or vaccinated against yellow fever, Japanese encephalitis, or tick-borne encephalitis, subsequent exposure to a related flavivirus can result in a rapid and brisk rise in neutralizing antibodies against multiple flaviviruses (20). In addition, the neutralizing antibody titer against a flavivirus to which the person previously was exposed might be higher than the titer against the virus with which they were most recently infected (20). For example, a person who was previously infected with dengue virus or who received yellow fever vaccine might respond with high levels of neutralizing antibodies against those viruses when later infected with Zika or West Nile viruses. When performing serologic testing, the presence of these neutralizing antibodies against multiple flaviviruses can preclude conclusive determination of which flavivirus was responsible for the recent infection.

Zika Virus Antibody Testing

An enzyme-linked immunosorbent assay (ELISA) can be used to detect anti-Zika virus IgM antibodies in serum or cerebrospinal fluid; however, the Zika virus IgM ELISA can provide false-positive results because of cross-reacting IgM antibodies against related flaviviruses or nonspecific reactivity. The plaque



reduction neutralization test (PRNT) measures virus-specific neutralizing antibody titers and should be performed against various related flaviviruses to rule out false-positive ELISA results. In primary flavivirus infections (i.e., the first time a person is infected with a flavivirus), PRNT also can be used to identify the infecting virus. Usually, this is determined with a neutralizing antibody titer ≥ 4 -fold higher than titers against cross-reacting flaviviruses. Based on earlier flavivirus research and limited preliminary data specific to Zika virus, the historical use of a 4-fold higher titer by PRNT might not discriminate between anti-Zika virus antibodies and cross-reacting antibodies in all persons who have been previously infected with or vaccinated against a related flavivirus (i.e., secondary flavivirus infection) (20,21). Because of the importance of appropriate clinical management of Zika and dengue virus infections, and the risk for adverse pregnancy outcomes in women infected with Zika virus during pregnancy, a conservative approach to the interpretation of antibody test results is now recommended to reduce the possibility of missing the diagnosis of either infection (9,11).

CDC Zika Virus Diagnostic Tests

The Food and Drug Administration (FDA) has issued an Emergency Use Authorization for the CDC Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) for antibody testing (3). This assay has been introduced and is being used in qualified public health and Department of Defense laboratories in the United States. The Zika MAC-ELISA is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid collected from persons meeting the clinical and epidemiologic criteria for suspected Zika virus disease (3,22). Results are reported as positive (termed “presumptive positive” to denote the need to perform a confirmatory PRNT), equivocal, negative, or inconclusive (i.e., results uninterpretable because of high background optical density). To resolve false-positive results that might be caused by cross-reactivity or nonspecific reactivity, presumptive positive results should be confirmed with PRNT against Zika, dengue, and other flaviviruses to which the person might have been exposed (3,23). In addition, equivocal and inconclusive results that are not resolved by retesting also should have PRNT performed to rule out a false-positive result.

Interpretation of Zika Virus Testing Results

For persons with suspected Zika virus disease, a positive rRT-PCR result confirms Zika virus infection, and no antibody testing is indicated (3,4,7). However, because of the decline in the level of viremia over time and possible inaccuracy in

Summary

What is already known about this topic?

Zika virus is a mosquito-borne flavivirus closely related to dengue, West Nile, Japanese encephalitis, and yellow fever viruses. Diagnostic testing for Zika virus infection can be accomplished using both molecular and serologic methods. However, results of Zika virus antibody testing can be difficult to interpret because of cross-reactivity with related flaviviruses, which can preclude identification of the specific infecting virus, especially when the person previously was infected with or vaccinated against a related flavivirus.

What is added by this report?

For persons with suspected Zika virus disease, a positive real-time reverse transcription–polymerase chain reaction (rRT-PCR) result confirms Zika virus infection, but a negative result does not exclude infection. In these cases, antibody testing can identify additional recent Zika virus infections. If immunoglobulin (Ig) M test results are positive, equivocal, or inconclusive, performing a plaque reduction neutralization test (PRNT) is needed to confirm the diagnosis. However, recent evidence suggests that a 4-fold higher titer by PRNT might not discriminate between anti-Zika virus antibodies and cross-reacting antibodies in all persons who have been previously infected with or vaccinated against a related flavivirus. Thus, a more conservative approach to interpreting PRNT results is now recommended to reduce the possibility of missing the diagnosis of either Zika or dengue virus infection.

What are the implications for public health practice?

All patients with clinically suspected dengue should receive appropriate management to reduce the risk for hemorrhagic medical complications. Pregnant women with laboratory evidence of a recent Zika virus infection or flavivirus infection should be evaluated and managed for possible adverse pregnancy outcomes and reported to the appropriate Zika virus pregnancy registry. Health care providers should consult with state or local public health authorities for assistance in interpreting test results.

reporting of dates of illness onset, a negative rRT-PCR result does not exclude Zika virus infection. Therefore, serum IgM antibody testing for Zika and dengue virus infections should be performed if rRT-PCR is negative. For serum specimens collected < 7 days after onset of symptoms, the combination of a negative rRT-PCR result and negative IgM antibody testing suggests that there was no recent infection. However, a negative IgM antibody test, in the absence of rRT-PCR testing, might reflect specimen collection before development of detectable antibodies and does not rule out infection with the viruses for which testing was performed. For specimens collected from 7 days to 12 weeks after onset of symptoms, a negative IgM antibody result to both Zika and dengue viruses rules out recent infection with either virus.

TABLE. Interpretation of results of antibody testing for suspected Zika virus infection^{*,†,§,¶,} — United States, 2016**

Zika virus and dengue virus IgM ELISA	Zika virus PRNT	Dengue virus PRNT	Interpretation
Positive or equivocal (either assay)	≥10	<10	Recent Zika virus infection
Positive or equivocal (either assay)	<10	≥10	Recent dengue virus infection
Positive or equivocal (either assay)	≥10	≥10	Recent flavivirus infection; specific virus cannot be identified
Inconclusive in one assay AND inconclusive or negative in the other	≥10	<10	Evidence of Zika virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	<10	≥10	Evidence of dengue virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	≥10	≥10	Evidence of flavivirus infection; specific virus and timing cannot be determined
Any result (either or both assays)	<10	<10	No evidence of Zika virus or dengue virus infection
Positive for Zika virus AND negative for dengue virus	Not yet performed		Presumptive recent Zika virus infection
Positive for dengue virus AND negative for Zika virus	Not yet performed		Presumptive recent dengue virus infection
Positive for Zika virus AND positive for dengue virus	Not yet performed		Presumptive recent flavivirus virus infection
Equivocal (either or both assays)	Not yet performed		Equivocal results
Inconclusive in one assay AND inconclusive or negative in the other	Not yet performed		Inconclusive results
Negative for Zika virus AND negative for dengue virus	Not indicated		No evidence of recent Zika virus or dengue virus infection

Abbreviations: ELISA = enzyme-linked immunosorbent assay; IgM = immunoglobulin M antibodies; PRNT = plaque reduction neutralization test.

* For persons with suspected Zika virus disease, Zika virus real-time reverse transcription–polymerase chain reaction (rRT-PCR) should be performed on serum specimens collected <7 days after onset of symptoms, and on urine specimens collect <14 days after onset of symptoms.

† In the absence of rRT-PCR testing, negative IgM or neutralizing antibody testing in specimens collected <7 days after illness onset might reflect collection before development of detectable antibodies and does not rule out infection with the virus for which testing was conducted.

§ Zika IgM positive result is reported as “presumptive positive” to denote the need to perform confirmatory PRNT.

¶ Report any positive or equivocal IgM Zika or dengue results to state or local health department.

** To resolve false-positive results that might be caused by cross-reactivity or nonspecific reactivity, presumptive positive Zika IgM results should be confirmed with PRNT titers against Zika, dengue, and other flaviviruses to which the person might have been exposed. In addition, equivocal and inconclusive results that are not resolved by retesting also should have PRNT titers performed to rule out a false-positive result.

If either the Zika or dengue virus IgM antibody testing yields positive, equivocal, or inconclusive results, PRNTs against Zika and dengue viruses (or other flaviviruses endemic to the region where exposure occurred) should be performed. A PRNT using a 90% cutoff value with a titer ≥10 (the typical starting serum dilution used to establish the presence of virus-specific neutralizing antibodies) against Zika virus, together with negative PRNTs (i.e., <10) against other flaviviruses is confirmatory for recent infection with Zika virus (Table). A PRNT titer ≥10 for both Zika and dengue virus (or another flavivirus) provides evidence of a recent infection with a flavivirus but precludes identification of the specific infecting virus. A negative PRNT against Zika virus in a specimen that is collected >7 days after illness onset rules out Zika virus infection. For specimens collected <7 days after onset of symptoms, the combination of a negative rRT-PCR and a PRNT titer <10 suggests that there was no infection with Zika virus. However, in the absence of rRT-PCR testing, a PRNT titer <10 might reflect specimen collection before development of detectable neutralizing antibodies and does not rule out infection with the viruses for which testing was conducted. Without confirmatory PRNTs, it is not possible to determine whether a presumptive positive IgM antibody result against Zika virus reflects recent flavivirus infection or a false-positive result.

For asymptomatic pregnant women residing in an area with local Zika virus transmission, IgM testing should be performed

upon initiation of prenatal care, mid-second trimester, and if any fetal abnormalities are detected during ultrasound evaluation (9). For asymptomatic pregnant women with a history of travel to areas where ongoing Zika virus transmission is occurring, Zika virus antibody testing should be performed on specimens collected 2–12 weeks post travel (9). Results are interpreted as for symptomatic persons. If a serum specimen was collected >12 weeks after travel, although IgM might still be present, it is possible that antibody levels have dropped below the detectable limit. Performing routine PRNTs for women in this group is not recommended because any result other than a PRNT titer <10 for Zika virus could represent infection with or vaccination against a flavivirus at any time in the past and does not provide specific evidence of Zika virus exposure during pregnancy.

Management of Persons with Suspected Zika or Dengue Virus Infection

All patients with clinically suspected dengue virus infection should receive appropriate management to reduce the risk for hemorrhagic complications (11). Symptomatic and asymptomatic pregnant women with serologic or molecular evidence of recent Zika virus infection should be evaluated and managed for possible adverse pregnancy outcomes and reported to the U.S. Zika Pregnancy Registry or the Puerto Rico Zika Active Pregnancy Surveillance System (9,10). Among

persons for whom serologic testing is unable to determine the most recent infecting flavivirus, an epidemiologic link to a laboratory-confirmed case of dengue or Zika virus disease can be considered in determining the most likely infecting virus (22). In addition, data on the epidemiology of viruses known to be circulating at the location of exposure and clinical features of these viral infections should be considered. If serologic testing is inconclusive or there is evidence of recent infection with either Zika or dengue virus, patients should be clinically managed for both infections because they might have been infected with either virus. Health care providers with questions about test result interpretation should consult with state or local public health authorities for assistance.

¹Zika virus response epidemiology and laboratory teams, CDC.

Corresponding author: Ingrid B. Rabe, irabe@cdc.gov, 970-221-6400.

References

- Faye O, Freire CCM, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20th century. *PLoS Negl Trop Dis* 2014;8:e2636. <http://dx.doi.org/10.1371/journal.pntd.0002636>
- Hayes EB. Zika virus outside Africa. *Emerg Infect Dis* 2009;15:1347–50. <http://dx.doi.org/10.3201/eid1509.090442>
- Food and Drug Administration. Zika virus emergency use authorization. Silver Spring, MD: US Department of Health and Human Services, Food and Drug Administration; 2016. <http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>
- CDC. Interim guidance for Zika virus testing of urine—United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:474. <http://dx.doi.org/10.15585/mmwr.mm6518e1>
- Bingham AM, Cone M, Mock V, et al. Comparison of test results for Zika virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika virus disease—Florida, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:475–8. <http://dx.doi.org/10.15585/mmwr.mm6518e2>
- Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* 2008;14:1232–9. <http://dx.doi.org/10.3201/eid1408.080287>
- CDC. Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US public health laboratories. <http://www.cdc.gov/zika/pdfs/denvchikvzika-testing-algorithm.pdf>
- Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships between flaviviruses as determined by cross-neutralization tests with polyclonal antisera. *J Gen Virol* 1989;70:37–43. <http://dx.doi.org/10.1099/0022-1317-70-1-37>
- Petersen EE, Polen KND, Meaney-Delman D, et al. Update: interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure—United States, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:315–22. <http://dx.doi.org/10.15585/mmwr.mm6512e2>
- Simeone RM, Shapiro-Mendoza CK, Meaney-Delman D. Possible Zika virus infection among pregnant women—United States and territories, May 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:514–9.
- World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control, 2009. Geneva, Switzerland: World Health Organization; 2009. http://apps.who.int/iris/bitstream/10665/44188/1/9789241547871_eng.pdf
- Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;360:2536–43. <http://dx.doi.org/10.1056/NEJMoa0805715>
- Musso D, Nhan T, Robin E, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill* 2014;19:20761. <http://dx.doi.org/10.2807/1560-7917.ES2014.19.14.20761>
- Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. *J Clin Virol* 2015;68:53–5. <http://dx.doi.org/10.1016/j.jcv.2015.04.021>
- Prince HE, Tobler LH, Yeh C, Gefer N, Custer B, Busch MP. Persistence of West Nile virus-specific antibodies in viremic blood donors. *Clin Vaccine Immunol* 2007;14:1228–30. <http://dx.doi.org/10.1128/CVI.00233-07>
- Roehrig JT, Nash D, Maldin B, et al. Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. *Emerg Infect Dis* 2003;9:376–9. <http://dx.doi.org/10.3201/eid0903.020531>
- Whitehead SS, Blaney JE, Durbin AP, Murphy BR. Prospects for a dengue virus vaccine. *Nat Rev Microbiol* 2007;5:518–28. <http://dx.doi.org/10.1038/nrmicro1690>
- Busch MP, Kleinman SH, Tobler LH, et al. Virus and antibody dynamics in acute West Nile virus infection. *J Infect Dis* 2008;198:984–93. <http://dx.doi.org/10.1086/591467>
- Poland JD, Calisher CH, Monath TP, Downs WG, Murphy K. Persistence of neutralizing antibody 30–35 years after immunization with 17D yellow fever vaccine. *Bull World Health Organ* 1981;59:895–900.
- Halstead SB, Rojanasuphot S, Sangkawibha N. Original antigenic sin in dengue. *Am J Trop Med Hyg* 1983;32:154–6.
- Johnson BW, Kosoy O, Martin DA, et al. West Nile virus infection and serologic response among persons previously vaccinated against yellow fever and Japanese encephalitis viruses. *Vector Borne Zoonotic Dis* 2005;5:137–45.
- Council of State and Territorial Epidemiologists. Zika virus disease and congenital Zika virus infection interim case definition and addition to the Nationally Notifiable Diseases list. Atlanta, GA: Council of State and Territorial Epidemiologists; 2016. https://www.cste2.org/docs/Zika_Virus_Disease_and_Congenital_Zika_Virus_Infection_Interim.pdf
- Martin DA, Muth DA, Brown T, Johnson AJ, Karabatsos N, Roehrig JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;38:1823–6.

Readers who have difficulty accessing this PDF file may access the HTML file at http://www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm?s_cid=mm6521e1_w. Address all inquiries about the *MMWR* Series, including material to be considered for publication, to Editor, *MMWR* Series, Mailstop E-90, CDC, 1600 Clifton Rd., N.E., Atlanta, GA 30329-4027 or to mmwrq@cdc.gov.